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## Isolation, Characterization, and Quantification of Steroidal Saponins in Switchgrass (*Panicum virgatum* L.)

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Switchgrass (*Panicum virgatum* L.) has been identified for development into an efficient and environmentally friendly biomass energy crop. A recent 5 year study demonstrated that switchgrass grown for biofuel production produced 540% more energy than what is needed to grow, harvest, and process it into cellulosic ethanol. If switchgrass is grown on a scale useful for a bioenergy source, some of the material could be used by livestock as hay or pasture. Switchgrass has been reported to cause hepatogenous photosensitization in lambs (*Ovis aries*) and horses (*Equus caballus*). In this study, a simple extraction and rapid reversed phase high-performance liquid chromatography–electrospray ionization–mass spectrometry method was developed for quantifying the major saponins in switchgrass samples. Differences in the relative concentrations of different saponins were observed between switchgrass cultivars and plant parts.

**KEYWORDS:** Switchgrass; saponins; biofuel; hepatic; photosensitization

### INTRODUCTION

The U.S. Department of Energy has identified switchgrass (*Panicum virgatum* L.) for development into an efficient and environmentally friendly biomass energy crop that could reduce the nation's dependence on foreign oil, curb emissions of carbon dioxide, and strengthen the U.S. farm economy. A recent 5 year study based on large scale field trial data demonstrated that switchgrass grown for biofuel production produced 540% more energy than what is needed to grow, harvest, and process it into cellulosic ethanol (1).

Presently, there are over 35 million acres enrolled in the Conservation Reserve Program (CRP), with many CRP contracts east of the 100th Meridian containing switchgrass. In addition, if switchgrass is grown on a large scale for cellulosic ethanol production, a 50 million gallon per year ethanol plant would require 625000 tons of switchgrass per year. Consequently, at the current 5 tons/acre yield, each 50 million gallon per year plant would need to be in close proximity to 125000 acres of switchgrass. These switchgrass acres would not only be used

as a bioenergy source, but a portion of these acres would likely be used for hay or pasture for livestock.

Many grasses in the *Panicum* genus have been reported to cause hepatogenous photosensitization in animals throughout the world (2). Glycosidic steroidal saponins have been found in some species of the *Panicum* genus, and these compounds have been suggested as one of the primary agents causing hepatogenous photosensitization in animals grazing these grasses (3–6). Additionally, it currently is not known what impact saponins will have on the cellulosic ethanol fermentation process.

In the summer of 1991, Puoli et al. (7) conducted grazing trials to evaluate the use of switchgrass (*P. virgatum* L. cv. Cave-in-Rock) in grazing systems for increased lamb production and reported hepatogenous photosensitization in weaned and suckling lambs. Of a total of 104 lambs, 17 showed photosensitization, and eight died. Hepatotoxicity was confirmed by changes in blood metabolites. In this report, mature ewes were not affected, and the authors noted that suckling lambs grazing on switchgrass the previous year were not affected.

In 2000, poisoning of five horses grazing in a switchgrass pasture in eastern Nebraska was reported to our laboratory. One horse developed liver disease and died. Serum enzyme levels of the other four horses were suggestive of hepatic damage. The four living horses were removed from the pasture, and after 3 months, all four horses recovered and appeared to have normal

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hepatic function. The switchgrass from this pasture contained saponins and the sapogenin, diosgenin (8). A subsequent feeding trial was conducted in which sheep, horses, and goats (*Capra hircus*) were fed switchgrass hay. Sheep and horses did not develop photosensitization or observable hepatic disease or damage, but some goats developed skin lesions consistent with hepatic photosensitivity (9).

The previous reports of switchgrass photosensitization demonstrate the sporadic occurrence of the disease. These reports indicate the need for additional study of the variation in concentration of saponins and/or the hepatotoxic components during different growth stages of the plant and in different switchgrass cultivars.

In this study, the structures of the three major saponins in switchgrass were elucidated via NMR and mass spectrometry. In addition, a simple extraction and rapid reversed phase high-performance liquid chromatography–electrospray ionization–mass spectrometry (HPLC-ESI-MS) method was developed for identifying and quantifying saponins in switchgrass. Three different saponins were identified, and the differences in saponin concentrations between switchgrass varieties and plant parts were determined.

## MATERIALS AND METHODS

**Plant Material.** Four switchgrass cultivars (Trailblazer, Cave-in-Rock, Summer, and Kanlow) were grown in replicated yield trials at the University of Nebraska Agricultural Research and Development Center near Mead, NE. Plots were fertilized with 112 kg N ha<sup>-1</sup> in the spring. Field replicates of each cultivar were hand-harvested to a 10 cm stubble height on July 3, 2002. Grass samples were transported to the laboratory and separated into leaf and stem components. The leaf and stem components for each switchgrass cultivar were dried at 55 °C to a constant weight, ground to pass a 2 mm screen in a Wiley mill, and shipped to the Poisonous Plant Research Laboratory for analysis.

**Extraction and Isolation of Major Switchgrass Saponins.** Crude saponin fractions from Cave-in-Rock and Kanlow cultivars were extracted and isolated as previously described (8). These saponin fractions were further purified by chromatography on a 30 cm × 1.8 cm silica gel column using a mobile phase of CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (65:35:10, v/v/v; bottom layer). Fractions (15 mL) from the column were collected and analyzed for saponins by ESI-MS. Fractions containing the saponins were combined and rotoevaporated to dryness. The purified saponin extracts were then isolated into individual saponins by semipreparative reversed phase HPLC. Saponin extract (500  $\mu$ L, 20 mg/mL in 90:10, 0.1% formic acid:acetonitrile, v:v) was injected onto a 250 mm × 10 mm Betasil C18 column (50  $\mu$ m, 125 Å) (Thermo Electron Co., Bellefonte, PA). Saponins were eluted over a gradient using a mobile phase consisting of 0.1% formic acid:acetonitrile A:B. Mobile phase B was increased from 10 to 40% over 20 min with a flow rate of 5 mL/min. Eluate from the HPLC column was split 9:1 with 4.5 mL/min diverted to collection vials and 0.5 mL/min flow entering a Thermo Finnigan (San Jose, CA) LCQ ion trap mass spectrometer via an ESI source. Full scan mass data were collected for a mass range of 300–1500 amu. Under these conditions, saponin A eluted at 17.0 min, saponin B eluted at 17.3 min, and saponin C eluted at 16.6 min. The mass spectrometer was used as a detector, allowing the collection of fractions (saponins A–C) from the diverted flow into collection vials (45 mL) filled with deionized distilled water (20 mL). The fractions containing the diluted samples were then passed through a 3 mL SUPELCLEAN LC-18 cartridge (Supelco, Bellefonte, PA), which was previously conditioned by washing with methanol (1 mL) and equilibrated with deionized distilled water (1 mL). After application of the sample to the cartridge, the cartridge was washed with 5% methanol in water (1 mL), and finally, the saponin was eluted from the cartridge with methanol (3 mL). The methanol was evaporated from the sample under a gentle flow of N<sub>2</sub> at 40 °C. Using this method, saponin A was isolated from Cave-in-Rock plant material and used

for structural characterization and standard material. Also using this method, saponin B and a 60:40 mixture of saponin C:saponin A were isolated for structural characterization from Kanlow plant material.

**Per-O-methylation.** The lyophilized sample was dissolved in dimethylsulfoxide and then methylated with NaOH and methyl iodide (10). The reaction was quenched by addition of water, and the per-O-methylated product was extracted with dichloromethane. The organic phase was concentrated to dryness and then dissolved in 50% methanol prior to analysis by MS.

**Direct Infusion ESI-MS<sup>n</sup>.** ESI-MS<sup>n</sup> spectra of the permethylated saponins were acquired on LCQ-Ion trap instrument (Thermo Finnigan), in the positive ion mode. The samples were infused at a constant flow rate of 3.0  $\mu$ L/min via syringe pump (Harvard Apparatus) and sprayed at 3.5 kV. The capillary temperature was set to 200 °C. A normalized collision energy of 40% and an isolation mass window of 2.2 Da were applied to obtain MS<sup>n</sup>.

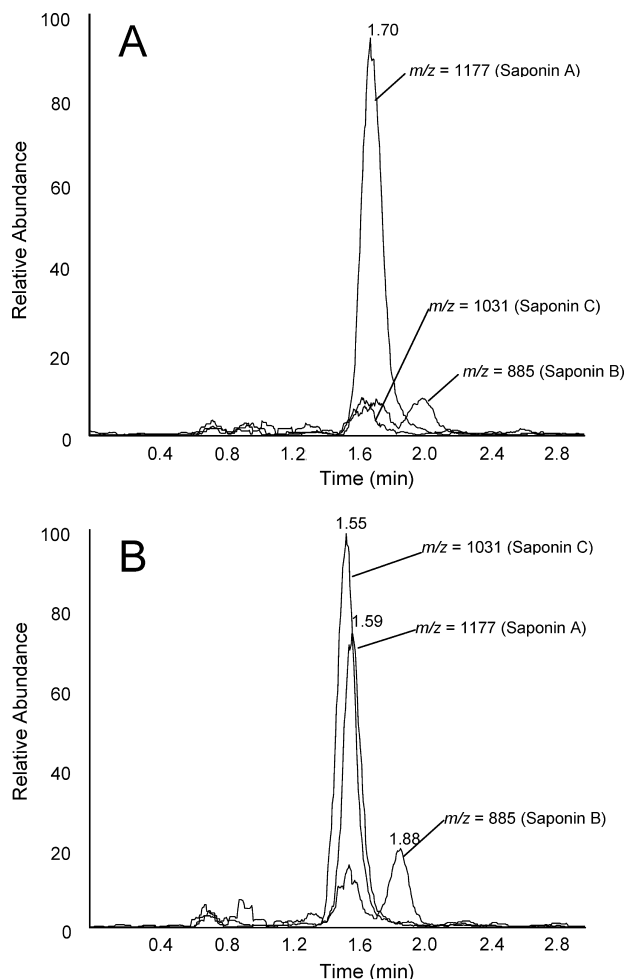
**NMR Spectroscopy.** The purified saponin was dissolved in 1 mL of CD<sub>3</sub>OD and dried in a stream of nitrogen. The sample was taken up in 0.7 mL of pyridine-*d*<sub>5</sub> and placed into a 5 mm NMR tube. One-dimensional proton, correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), nuclear Overhauser enhancement spectroscopy (NOESY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) NMR spectra were acquired on a Varian Inova-800 MHz spectrometer at 298 K (25 °C). Chemical shifts were measured relative to one of the pyridine signals ( $\delta_H$  = 7.22 ppm,  $\delta_C$  = 123.9 ppm). COSY was acquired with 800 increments, four scans per increment. Increments and scans for the other experiments were 256\*8 (TOCSY), 128\*16 (NOESY), 128\*128 (HSQC), and 100\*512 (gHMBC). Mixing times were 100 and 300 ms for TOCSY and NOESY, respectively.

**Analytical Scale Extraction.** Extraction of switchgrass plant material for saponin analysis was accomplished by weighing 100 mg of ground switchgrass plant material into a 13 mL screw top test tube equipped with Teflon lined caps (Pierce, Rockford, IL). Methanol (MeOH, 5 mL) was added to each test tube and placed in a mechanical shaker for 30 min and then centrifuged to separate the plant residue and MeOH extract. The MeOH extract was transferred to a clean 20 mL test tube. The switchgrass residue was extracted two more times with 5 mL of MeOH for 30 min, and all MeOH extracts were combined for a total of 15 mL. A 0.5 mL aliquot was transferred to a 7 mL vial and evaporated to dryness on a heat block at 65 °C under a gentle flow of nitrogen. The dried aliquot was then reconstituted in 1.0 mL of 0.1% formic acid:acetonitrile (90:10). The sample was then passed through a 0.20  $\mu$ m syringe filter (National Scientific, Rockwood, TN), and 0.5 mL was then transferred to a 1 mL autosample vial for analysis.

**HPLC-ESI-MS.** Samples were injected (5  $\mu$ L) onto a Betasil C-8 reversed phase column (100 mm × 2.1 mm i.d.) (Thermo Electron Corp., Waltham, MA) protected by a guard column of the same phase. The saponins were eluted from the column with an isocratic flow (0.500 mL/min) of 72:28 (0.1% formic acid:acetonitrile) mobile phase. The total HPLC run time was 3.0 min. Flow from the column was connected directly to a Thermo Finnigan LCQ ion trap mass spectrometer via an ESI source. Full scan mass data were collected for a mass range of 300–1300 amu. MS/MS product ion spectra were collected after isolation of a selected precursor ion ( $\pm 5$  amu), and the relative collision energy was manually adjusted to observe significant fragmentation of the selected ion. Saponin A, isolated by preparative reversed phase HPLC, was used as a standard to quantify saponins A–C. Saponin A standard was prepared in a solution of 0.1% formic acid:acetonitrile (90:10) to give a six point standard curve over the range of 1.56–50.0  $\mu$ mg/mL by serial dilution. Peak areas of the individual saponins (saponins A–C) were determined from reconstructed ion chromatograms of the respective MH<sup>+</sup> – H<sub>2</sub>O ions ( $m/z$  = 1177, 885, and 1031).

## RESULTS AND DISCUSSION

**Identification of Switchgrass Saponins.** The extracted switchgrass saponin material was analyzed by reversed phase HPLC-ESI-MS. Three major saponin components were detected from the HPLC and MS data. The HPLC peak eluting at 1.70 min in Figure 1A and 1.59 min in Figure 1B was, in general,



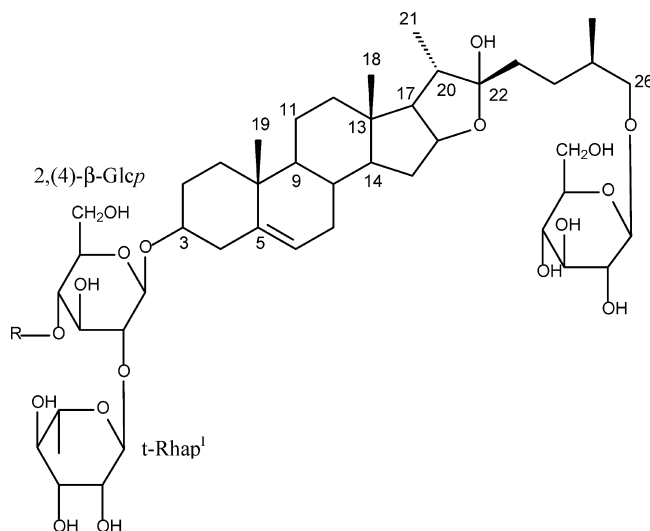
**Figure 1.** Reconstructed ion chromatograms ( $m/z = 1177$ ,  $1031$ , and  $885$ ) from (A) Trailblazer leaf and (B) Kanlow leaf.

**Table 1.** Measured Amounts of Total and Individual Saponins A–C from Plant Parts of Switchgrass Varieties Trailblazer, Cave-in-Rock, Summer, and Kanlow

cultivar	sample	saponins (mg/100 mg plant)			
		A (mg)	B (mg)	C (mg)	total (mg)
Trailblazer	total	0.26	0.03	0	0.29
Trailblazer	leaf	0.56	0.06	0	0.62
Trailblazer	stem	0.15	0.03	0	0.18
Cave-in-Rock	total	0.24	0.04	0	0.28
Cave-in-Rock	leaf	0.63	0.06	0.02	0.71
Cave-in-Rock	stem	0.06	0.02	0	0.08
Summer	total	0.30	0.02	0.02	0.34
Summer	leaf	0.55	0.02	0.05	0.62
Summer	stem	0.05	0.01	0.01	0.07
Kanlow	total	0.11	0.06	0.18	0.35
Kanlow	leaf	0.34	0.09	0.41	0.84
Kanlow	stem	0.02	0.07	0.09	0.18

the major saponin for all four switchgrass varieties (Table 1). The ESI-MS spectrum of this saponin showed a  $MH^+ - H_2O$  ion at  $m/z = 1177$ . The component was labeled as saponin A. A second saponin peak (saponin B) eluting at 1.88 min ( $MH^+ - H_2O$ ,  $m/z = 885$ ) was also found in all four switchgrass varieties (Table 1 and Figure 1A,B). A third saponin peak, saponin C ( $MH^+ - H_2O$ ,  $m/z = 1031$ ), which essentially coeluted with saponin A, was present in the Summer and Kanlow switchgrass varieties (Table 1 and Figure 1A,B).

The composition of the aglycone portion of the saponins was determined after hydrolysis of the saponins and analysis by gas



Saponin A:  $R = \alpha\text{-Rhap}^{\text{II}}\text{-}1 \rightarrow 4\text{-}\alpha\text{-Rhap-}$

Saponin B:  $R = \text{H}$

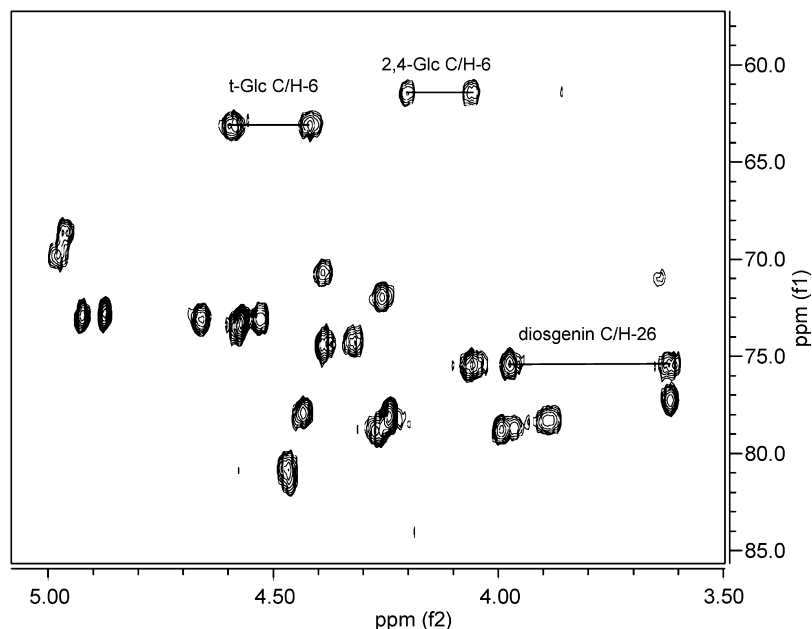
Saponin C:  $R = \alpha\text{-Rhap}^{\text{II}}$

**Figure 2.** Structures of saponins A–C.

chromatography–mass spectrometry (GC-MS) as previously described (8). Diosgenin was observed in all four switchgrass varieties as the major aglycone.

Further characterization of the attached glycosidic residues of the saponins was accomplished by monosaccharide analysis and methylation analysis using GC-MS, mass spectrometry, and NMR spectroscopy. Monosaccharide analysis of saponin A revealed the presence of glucose and rhamnose in a molar ratio of 44:56. In addition, methylation analysis showed terminal rhamnose, 4-linked rhamnose, terminal glucose, and 2,4-linked glucose for saponin A.

ESI-MS of the permethylated saponin A gave a singly charged ion at  $m/z = 1427.85$  and a doubly charged ion at  $m/z = 725.61$ . This mass did not agree with the possible structures consistent with the linkage data, making it necessary to elucidate the structure by NMR. An HSQC experiment with multiplicity editing, which gives methyl and methine groups as positive peaks and methylene groups as negative peaks, showed three sets of methylene signals in the carbohydrate ring region. This region contains carbons that are directly attached to one oxygen atom. Two of the methylene signals clearly belonged to the two glucose residues, but the third had to arise from the aglycone, as there are no more methylenes in the glycan portion of the saponin. C-26 is the only methylene group that is directly attached to oxygen in diosgenin, the putative aglycone of this saponin. However, the chemical shift of diosgenin C-26 has been reported to be around 67 ppm (11). The chemical shift found for C-26 in saponin A was 75.5 ppm, a downfield shift, which would be consistent with glycosylation at this position (Figure 3). The open and glycosylated F ring of diosgenin is also in agreement with the mass spectrum of the permethylated saponin A. The carbon chemical shifts of the carbohydrate portion of the molecule indicate the presence of two terminal rhamnoses, a 4-linked rhamnose, a 2,4-linked glucose, and a terminal glucose. NOESY indicates linkage of one terminal rhamnose to the 4-linked rhamnose. The other terminal rhamnose and the 4-linked rhamnose show an NOE to the 2,4-linked glucose, which in turn has an NOE to H-3 of the aglycone. That leaves only the terminal glucose to be attached to O-26 of



**Figure 3.** Partial HSQC spectrum of *O*-22-deuteromethylated saponin A with multiplicity editing. Annotated peaks are negative (methylene signals); other peaks are positive (methine signals).

**Table 2.** Chemical Shift Assignments of the Oligosaccharide Moiety of Saponin A (Pyridine- $d_5$ )

residue		chemical shift (ppm)					
		1	2	3	4	5	6
<i>t</i> - $\alpha$ -Rha <sup>I</sup>	<sup>1</sup> H	6.43	4.88	4.66	4.39	4.98	1.79
	<sup>13</sup> C	102.4	72.8	73.1	74.5	69.8	19.2
<i>t</i> - $\alpha$ -Rha <sup>II</sup>	<sup>1</sup> H	6.31	4.93	4.53	4.33	4.39	1.63
	<sup>13</sup> C	103.5	73.3	73.4	74.7	71.0	19.2
4- $\alpha$ -Rha	<sup>1</sup> H	5.87	4.57	4.58	4.47	4.96	1.63
	<sup>13</sup> C	102.8	73.2	73.5	81.4	68.9	19.2
2,4- $\beta$ -Glc	<sup>1</sup> H	4.97	4.24	4.25	4.44	3.62	4.21/4.07
	<sup>13</sup> C	100.4	78.7	78.2	77.9	77.2	61.8
<i>t</i> - $\beta$ -Glc	<sup>1</sup> H	4.87	4.06	4.27	4.25	4.00	4.60/4.43
	<sup>13</sup> C	105.6	75.7	78.8	72.0	78.8	63.4

the aglycone. Accordingly, we propose the saponin to have the structure pictured in **Figure 2**. This structure is in agreement with all the MS data as well, as outlined below. **Tables 2** and **3** list the NMR chemical shift assignments of this structure. It should be noted that the NMR structure is that of the *O*-22 deuteromethylated saponin. The deuteromethyl group is an artifact of the proton-deuterium exchange done by evaporation from deuterated methanol. We could observe the rapid exchange of the substituent on *C*-22 by dissolving the saponin in methanol, deuteromethanol, or water and recording ESI mass spectra. This type of behavior has been observed previously (12).

The monoisotopic mass of the permethylated saponin A is 1404.85, which is consistent with  $m/z$  1427.85 ( $M + Na$ , singly charged) and  $m/z$  725.61 ( $M + 2Na$ , doubly charged) observed in the ESI-full mass spectrum of permethylated saponin A (**Figure 4**). The tandem mass spectra on the permethylated saponin are also shown in **Figure 4**. The MS/MS of the parent ion 1428 only gave one fragment, at  $m/z$  1396, resulting from the loss of methanol. The MS<sup>3</sup> spectrum of  $m/z$  1396 and MS<sup>4</sup> of  $m/z$  1207 indicated the presence of two terminal rhamnoses, one internal rhamnose, and one terminal glucose-diosgenin fragment, all in agreement with the structure presented in **Figure 2**. The  $m/z$  1177 observed in LC-MS is due to dehydration of saponin A during ionization. The *C*-26-glycosylated diosgenin is converted to diosgenin during acid hydrolysis by closing of

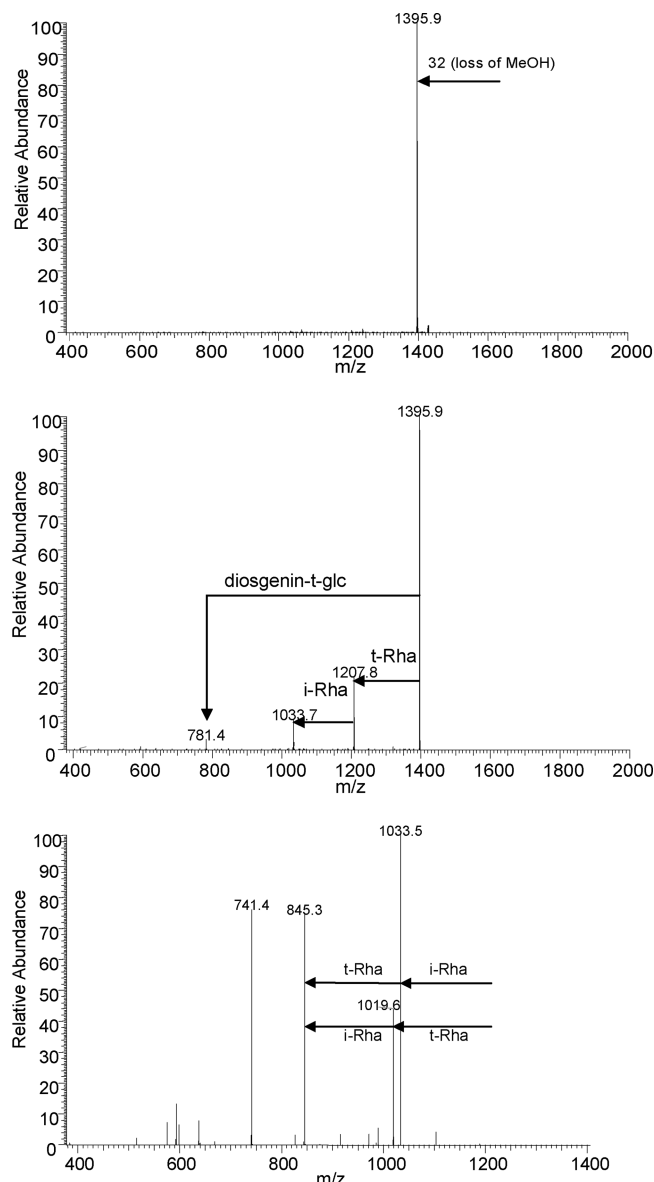
**Table 3.** Chemical Shift Assignments of the Aglycone Portion of Saponin A (Pyridine- $d_5$ )

position	<sup>1</sup> H (ppm)	<sup>13</sup> C (ppm)
1	1.76/1.00	37.8
2	2.09/1.87	30.7
3	3.89	78.3
4	2.81/2.74	39.3
5		141.9
6	5.34	121.2
7	1.88/1.48	32.7
8	1.56	32.2
9	0.89	50.6
10		37.7
11	1.44/1.41	21.5
12	1.69/1.06	40.0
13		41.9
14	1.04	56.8
15	1.99/1.43	32.5
16	4.47	80.7
17	1.76	64.6
18	0.89	14.7
19	1.07	19.8
20	2.23	40.7
21	1.21	16.8
22 <sup>a</sup>		113.6
23	2.01/1.77	31.2
24	1.81/1.35	28.6
25	1.91	34.5
26	3.97/3.63	75.5
27	1.02	17.7

<sup>a</sup> *C*-22 is substituted with  $-OCD_3$ , an artifact of the sample preparation for NMR.

the F ring and is thusly detected by GC-MS. The structures of saponin A determined here by MS/MS analysis of the permethylated products and NMR analysis of the intact saponin correct the previously proposed structure, which was based on the ring-closed diosgenin aglycone model (8).

These data and the resulting structure (**Figure 2**) agree with the saponin named dichotomin [(25*R*)-furost-5-ene-3 $\beta$ ,22 $\alpha$ ,26-triol 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl 26-*O*- $\beta$ -D-glucopyranoside] isolated from *Panicum dichotomiflorum* and *Allium ascalonium* L. (6, 13).



**Figure 4.** Tandem mass spectra of saponin A. (A) MS<sup>2</sup> on ion 1428, (B) MS<sup>3</sup> on ion 1396, and (C) MS<sup>4</sup> on ion 1207.

Comparison of the HSQC spectrum of saponin B with that of saponin A is evidence of the lack of the two rhamnose residues comprising the side chain attached to *O*-4 of the glucose linked to *O*-3 of the aglycone. COSY, TOCSY, and HSQC of saponin B clearly showed that same glucose to be only 2-linked, consistent with the structure (25*R*)-furost-5-ene-3 $\beta$ ,22 $\alpha$ ,26-triol 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucopyranosyl 26-*O*- $\beta$ -D-glucopyranoside, shown in **Figure 2**. The chemical shifts of the remaining sugar residues are essentially the same as in saponin A (see **Table 2**). The ESI mass spectrum of permethylated saponin B shows a peak at *m/z* 1079.51, which corresponds to the sodium adduct of the structure proposed by NMR analysis. The MS/MS spectrum of *m/z* 1080 only showed a peak at *m/z* 1047 due to loss of methanol. The MS<sup>3</sup> spectrum of *m/z* 1048 pointed out the presence of one terminal rhamnose, one internal glucose, and one terminal glucose-diosgenin fragment. This structure (**Figure 2**) is consistent with a saponin isolated from the subterranean part of *Ophiopogon planiscapus* NAKAI (14).

The HSQC of saponin C, which was contaminated with saponin A, was similar to the HSQC of pure saponin A in appearance but differed from it by the fact that the integration did not give the same values for all anomeric signals. The signal

corresponding to *t*- $\alpha$ -Rha<sup>II</sup> in saponin A was reduced in intensity, indicating that this residue is missing in saponin C. However, the anomeric peak of *t*- $\alpha$ -Rha<sup>I</sup> was also slightly reduced relative to the glucose residues, which may indicate that saponin C could be a mixture of isomers. MS/MS analysis of the permethylated saponin C sample was undertaken to answer this question. The ESI-MS full spectrum of permethylated saponin C showed a peak at *m/z* 1253.57, in addition to the signal at *m/z* 1427.68 arising from contaminating saponin A. The mass of saponin C indicates a glycan consisting of two glucose and two rhamnose residues. The MS/MS spectrum of 1254 only showed an ion at *m/z* 1221.57, arising from loss of methanol. The MS<sup>3</sup> spectrum of 1222 gave an ion at *m/z* 1033.46, coming from cleavage of terminal rhamnose. The MS<sup>4</sup> spectrum of 1033 again gave the loss of terminal rhamnose (*m/z* 845.33), giving strong evidence that saponin C is (25*R*)-furost-5-ene-3 $\beta$ ,22 $\alpha$ ,26-triol 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 4)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl 26-*O*- $\beta$ -D-glucopyranoside (**Figure 2**). This saponin named protodioscin has been isolated from several different plant species including puncturevine (*Tribulus terrestris*) and asparagus (*Asparagus officinalis*) (15, 16).

**Measured Saponin Concentration in Switchgrass Cultivars.** Saponins A–C were each quantified against a six point standard curve using isolated saponin A as the calibration standard. A correction to the peak areas measured for saponin C was needed because saponin A and saponin C coelute and because the ion at *m/z* 1031 may result from either saponin C (MH<sup>+</sup> – H<sub>2</sub>O) or a fragment ion of saponin A (MH<sup>+</sup> – H<sub>2</sub>O-146). The contribution of the latter would be subtracted from the peak area determined for saponin C. The relative average percent peak area of the 1031 ion was 7.8% as compared to the peak area for 1177 ion when averaged from the six point standard curve using saponin A. For the correction, the relative average percent area plus two standard deviations was 10.5% and was thus subtracted from the integrated 1031 peak areas to obtain corrected 1031 peak areas.

The data in **Table 1** show that in all tested switchgrass cultivars, the total saponin concentrations were highest in the leaves, lowest in the stems, and intermediate for the combined leaf and stem samples. The highest saponin concentrations were 0.84% of the total plant mass in leaves. In comparing switchgrass cultivars, the concentrations of saponins A–C and total saponins in the leaf, stem, and combined parts in the cultivars Trailblazer and Cave-in-Rock are very similar with saponins A and B, the major saponins, and little or no saponin C. In contrast, Summer, in addition to saponins A and B, has low but measurable amounts of saponin C in the leaf, stem, and combined parts. Among the four tested switchgrass cultivars, Kanlow was the most distinct in its saponin profile. In the Kanlow leaf, stem, and combined samples, the concentration of saponin C was higher than saponin A.

Switchgrass is a polymorphic species with two distinct ecotypes, lowland and upland, and two ploidy levels, tetraploid (36 chromosomes) and octaploid (72 chromosomes) (17). Lowland ecotypes are found on flood plains and other areas that receive run-on water, whereas upland ecotypes occur in upland areas that are not subject to inundation (17). The differences in saponins across cultivars may be explained by differences in ecotype and ploidy level. Trailblazer and Cave-In-Rock are upland octaploids, Summer is an upland tetraploid, and Kanlow is a lowland tetraploid. Trailblazer and Cave-In-Rock (upland octaploids) had little or no measurable saponin C, Summer (upland tetraploid) had intermediate concentrations of saponin C, and Kanlow (lowland tetraploid) had the highest

concentrations of saponin C (**Table 1**). Additionally, Trailblazer, Cave-In-Rock, and Summer had similar total concentrations of saponin B, but total concentrations of saponin B in Kanlow were higher than the other cultivars. Further study is warranted to determine why these concentrations differ across ecotype and ploidy level.

Here, we describe the structures of the three major saponins from the switchgrass samples evaluated in this study. We developed a simple extraction and rapid analysis method to quantify the saponins in plant material. We examined four switchgrass cultivars and found that the concentration of individual and total saponins differed among cultivars and plant parts. In all four switchgrass cultivars analyzed in this study, the leaves had the highest and stems had the lowest saponin concentrations. In addition, the saponin profile of Kanlow was unique as compared to the other three varieties.

The quantitation of saponins in switchgrass is important considering the current hypothesis that saponins are a primary agent causing hepatic photosensitization in animals grazing on switchgrass and that switchgrass has been identified for development into a biofuel source for the United States that could reduce the nation's dependence on foreign oil. If switchgrass is grown on a scale large enough for biofuel production, it is probable that some switchgrass land may be used for hay or pasture for livestock. In addition, some switchgrass byproducts from biofuel production may find their way into livestock feed. Until more is known about the toxicity of switchgrass, switchgrass breeders and growers should be cognizant of saponin levels in the different varieties of switchgrass that they are developing and using. Also, livestock producers should be aware of the risks and benefits of utilizing switchgrass or switchgrass byproducts for animal feed.

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